

Figure 1A

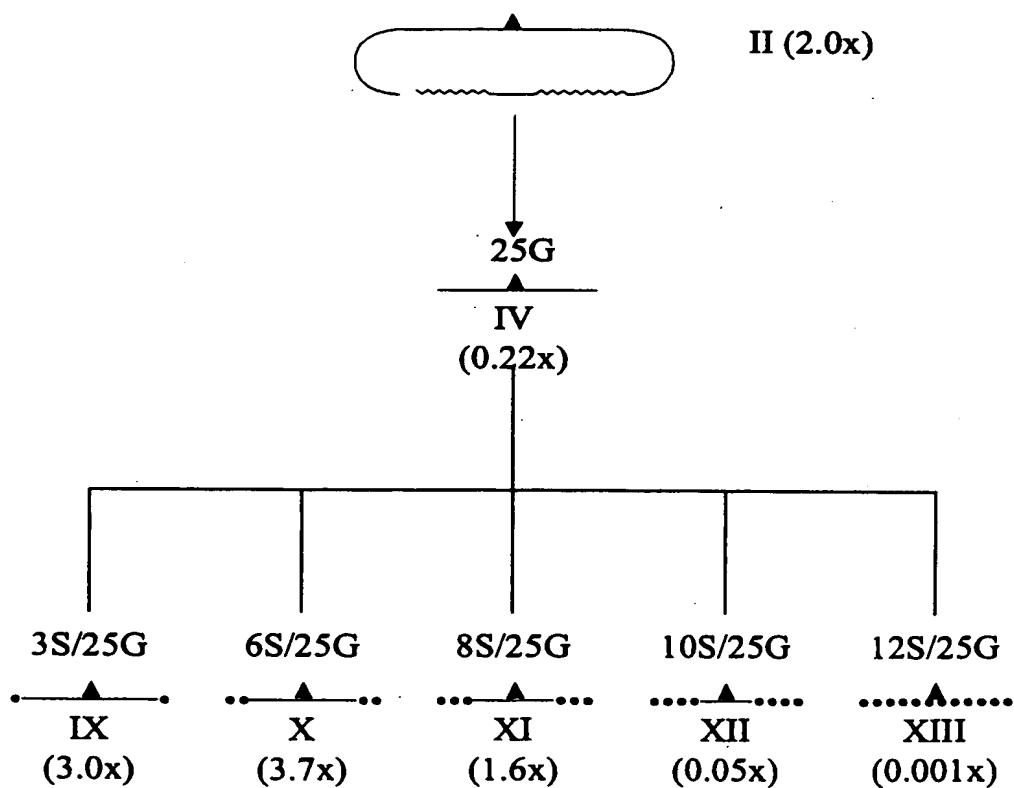


Figure 1B

EI124983108US

Plasmids, DNA targets and chimeric oligonucleotides

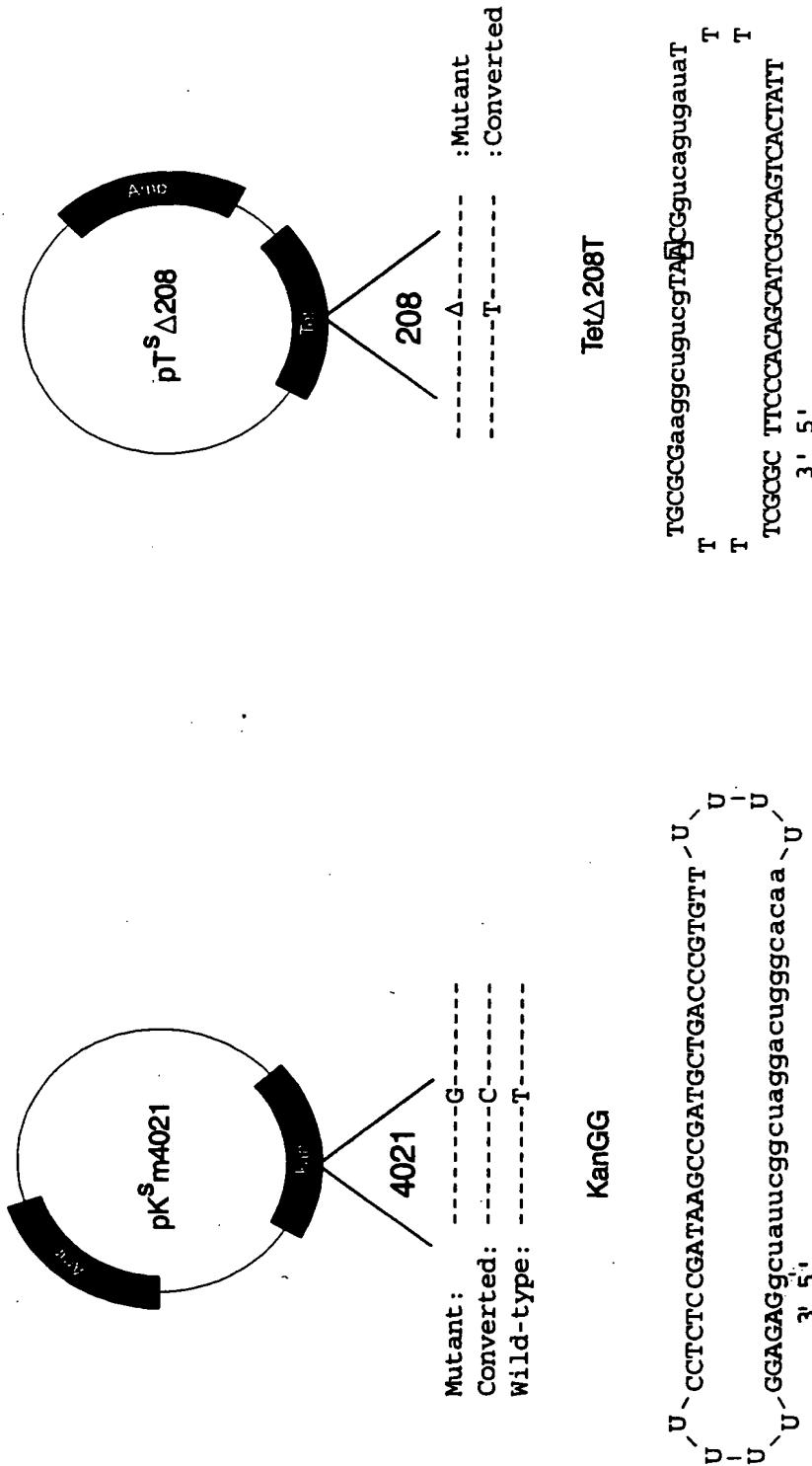


Figure 1C

2

TTGGCTA G GACTGG
AAGCCGAT C CTGACC

Kan mutant sequence

+

TTGGGCTA C GACTGG
AAGCCGAT G CTGACC

Kan converted sequence

DNA SEQUENCE ANALYSES

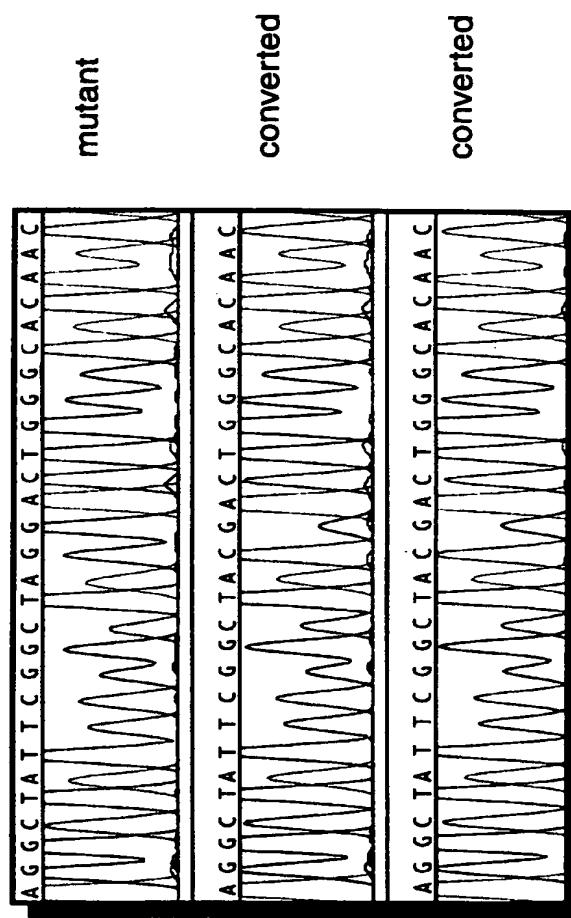


Figure 1D

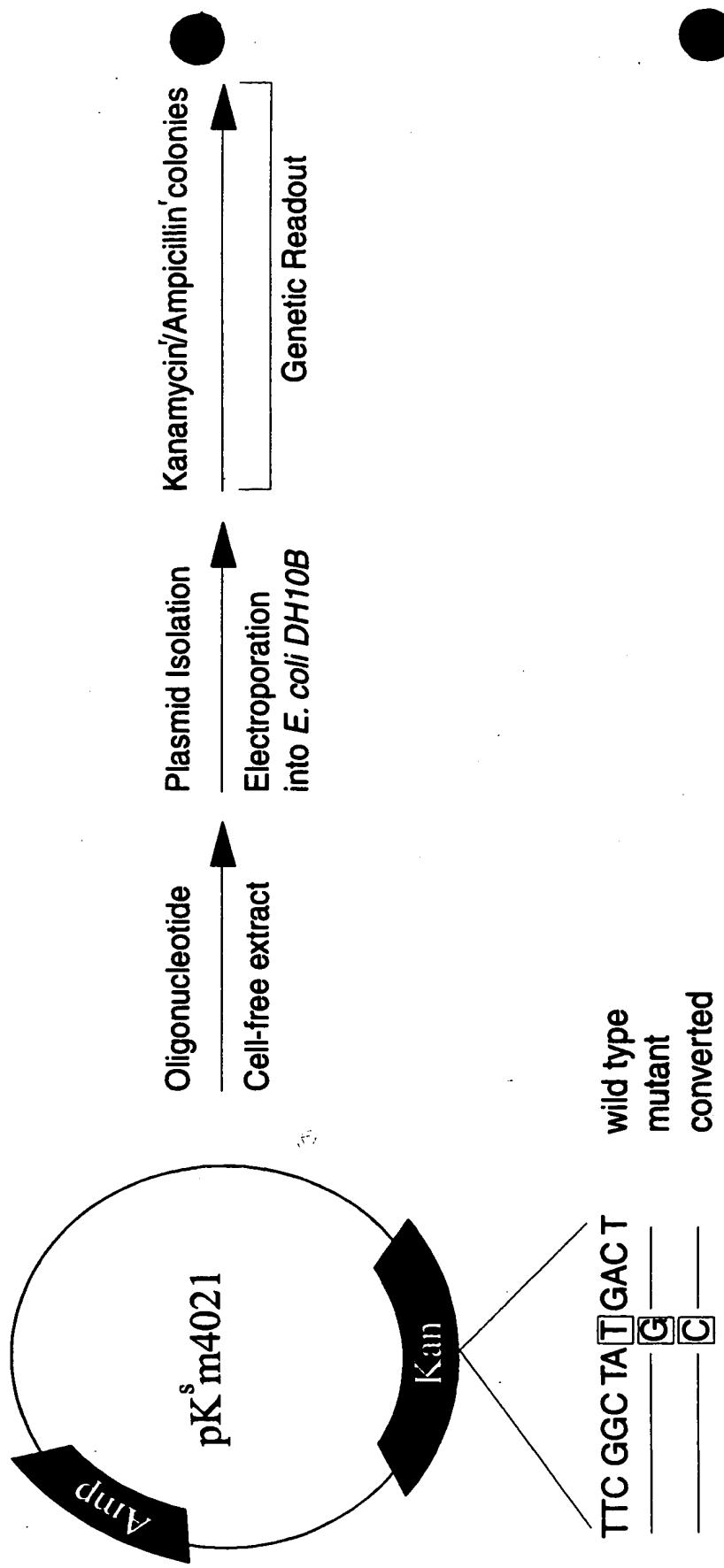
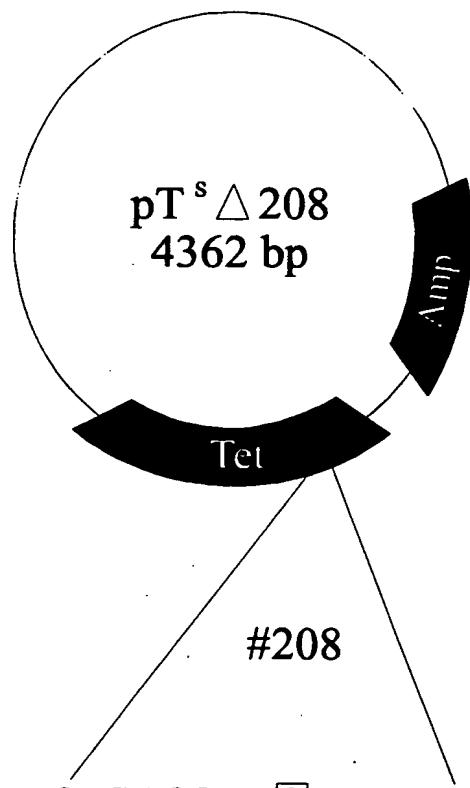


Figure 2



Wild type: GAC AGC AT **C** GCCAGT
 Mutant: GAC AGC AT - GCCAGT
 Converted: GAC AGC AT **T** GCCAGT

Sequence analysis of Tet^r plasmid $\Delta 208$

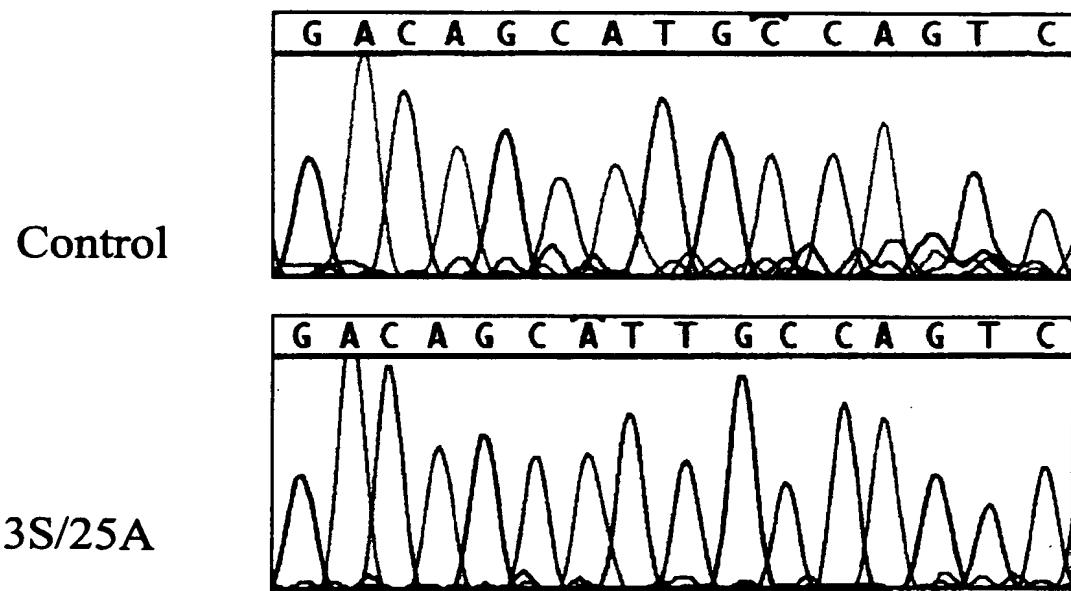
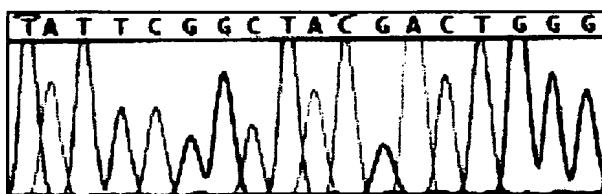


Figure 3

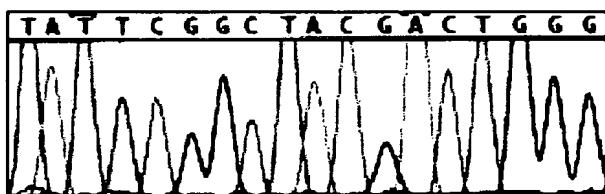
DNA sequence analysis of Kan^r plasmids

Target codon distribution					
oligomer	TAG	TAC	TAC/TAG	TGG	TCG
1) 3S/25G (20)	----	+	---	---	---
2) 6S/25G (20)	----	+	---	---	---
3) 8S/25G (20)	----	+	---	---	---
4) 10S/25G (18)	----	+	---	+(2)	+(2)
5) 25S/25G (4)	----	---	+ (2)	+ (2)	---

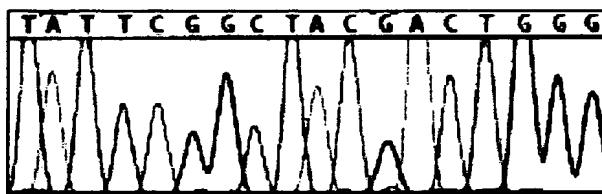
3S/25G



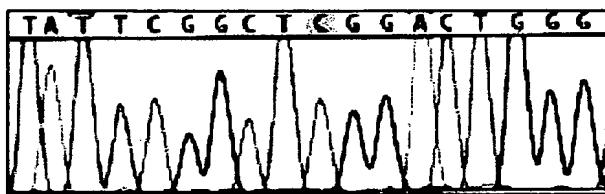
6S/25G



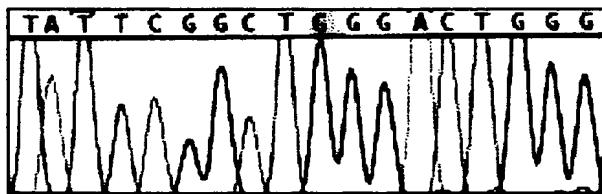
8S/25G



10S/25G



25S/25G



25S/25G



Figure 4

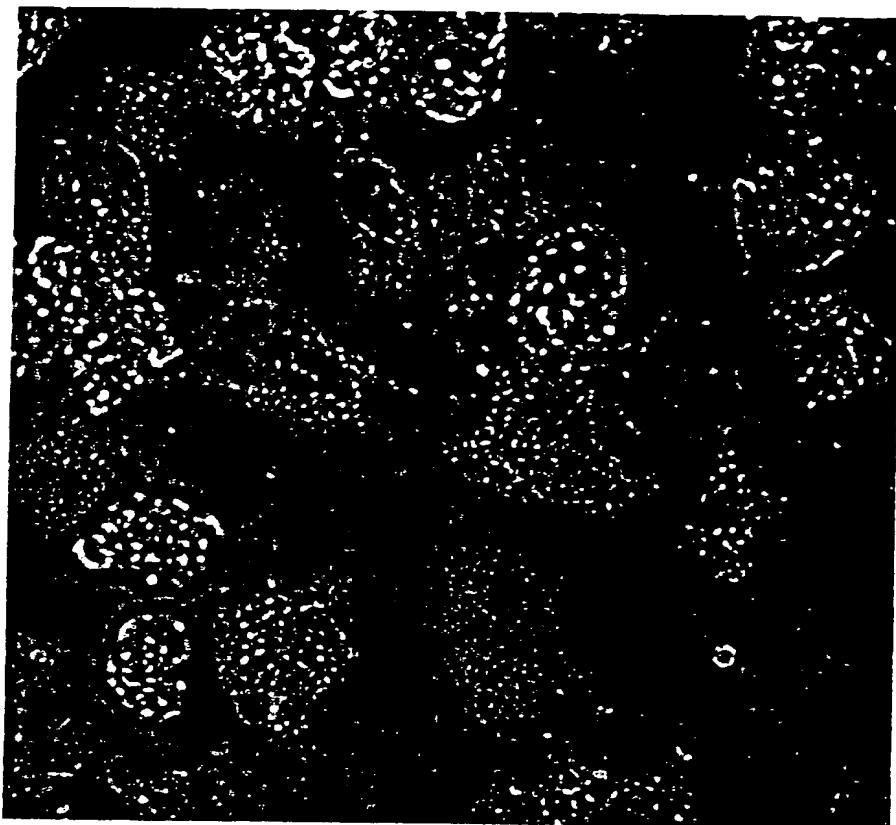


Figure 5

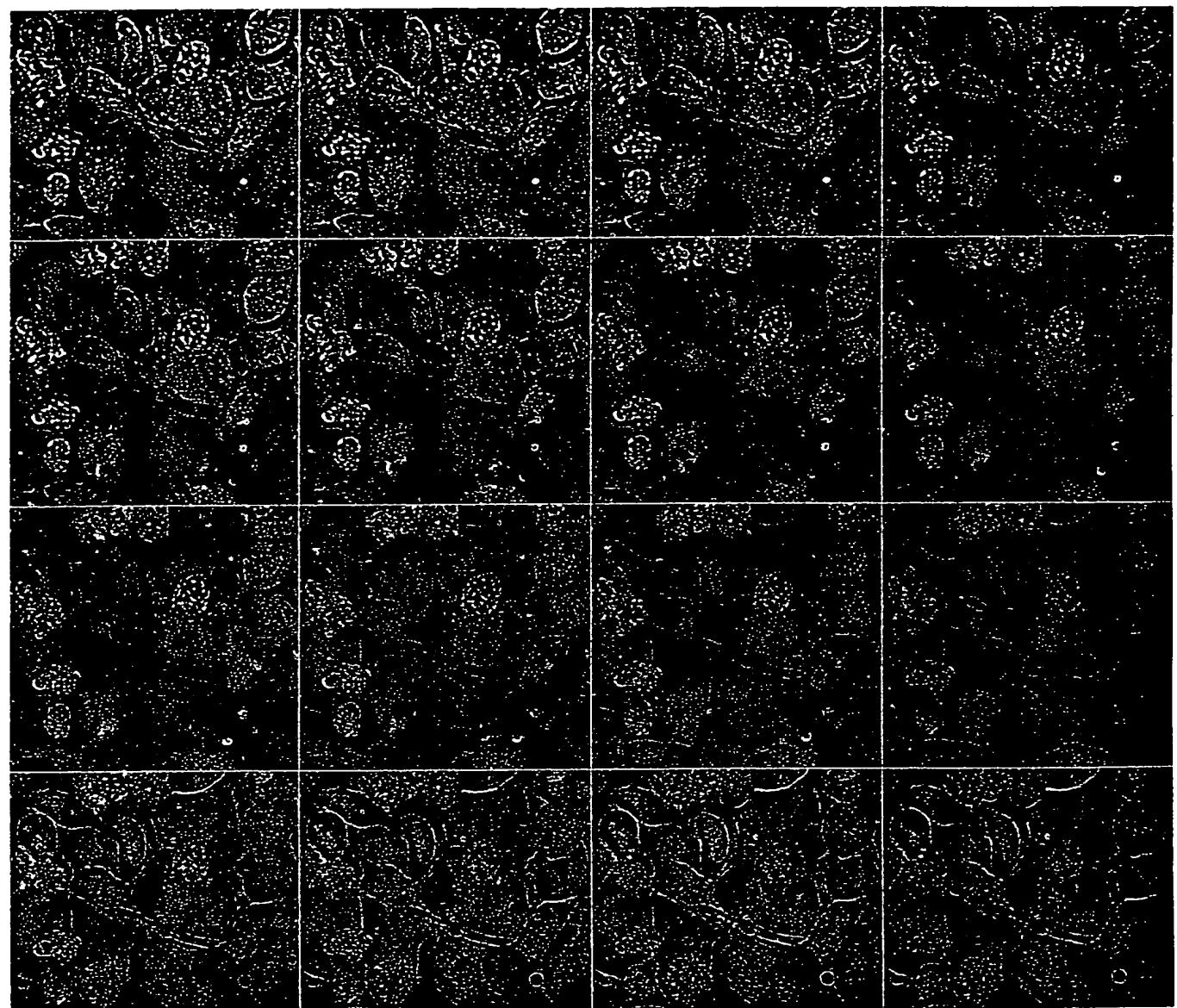


Figure 6

Sequence of normal allele: GTGGATATGTCCCT
Target/existing mutant: GTGGATAATGTCCCT
Desired alteration: GTGGATACGTCCCT

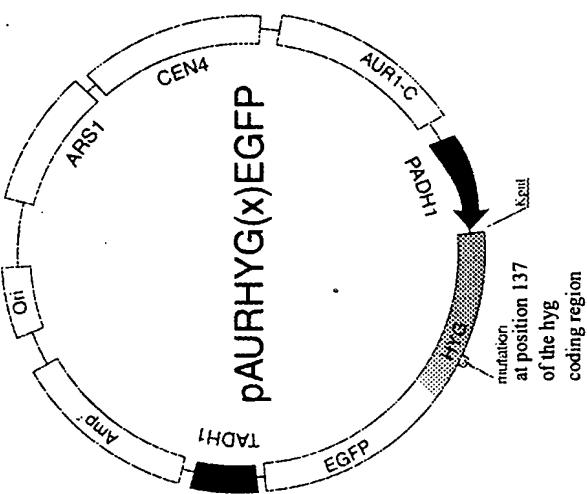


Figure 7A

Sequence of normal allele: GTGGATATGTCCCT
Target/existing mutant: GTGGATAGGTCCCT
Desired alteration: GTGGATACGTCCCT

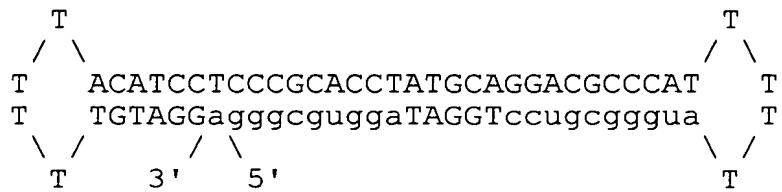
Figure 7B

HygE3T/25: 5'-AGG GCG TGG ATA CGT CCT GCG GGT A-3'

HygE3T/74: 5'-CTC GTG CTT TCA GCT TCG ATG TAG GAG GGC
GTG GAT ACG TCC TGC GGG TAA ATA GCT GCG
CCG ATG GTT TCT AC-3'

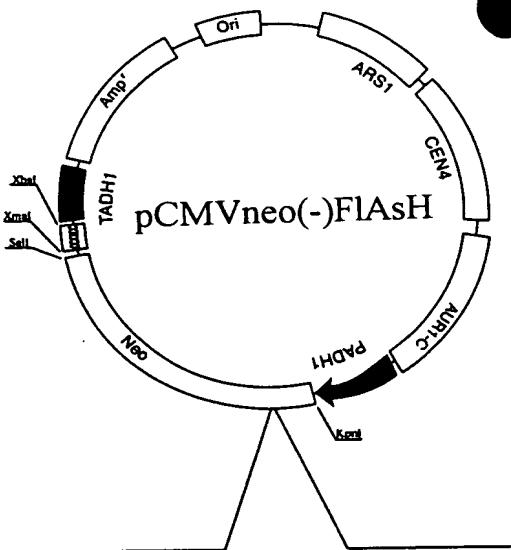
HygE3T/74α: 5'-GTA GAA ACC ATC GGC GCA GCT ATT TAC CCG
CAG GAC GTA TCC ACG CCC TCC TAC ATC GAA
GCT GAA AGC ACG AG-3'

HygGG/Rev:



Kan70T: 5'-CAT CAG AGC AGC CAA TTG TCT GTT GTG CCC AGT
CGT AGC CGA ATA GCC TCT CCA CCC AAG CGG CCG GAG
A-3'

Figure 8



TTCGGCTA G GACTGG
AAGCCGAT C CTGACC

Neo/kan target mutant

TTCGGCTA C GACTGG
AAGCCGAT G CTGACC

converted

FUSION GENE FOR LIGAND BINDING

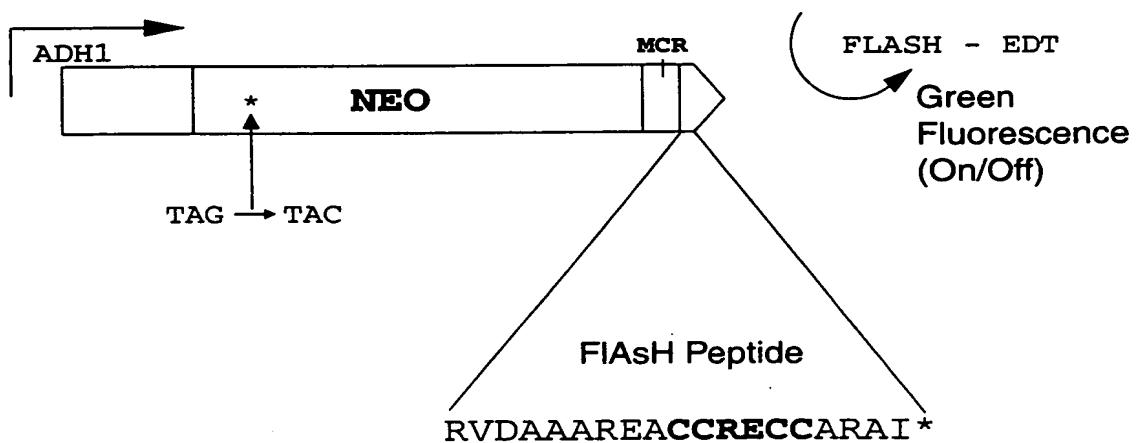


Figure 9

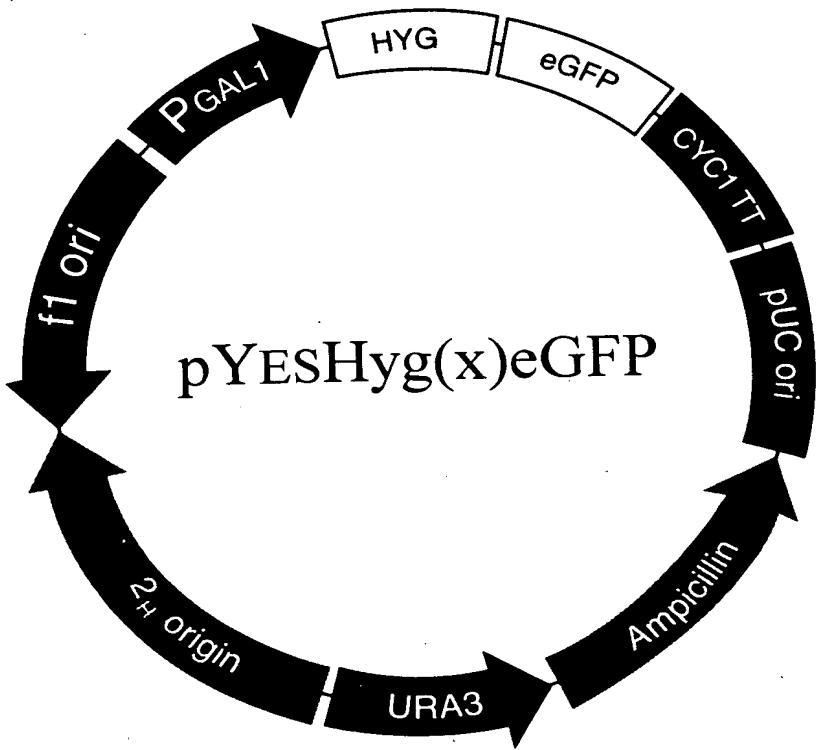


Figure 10